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Self-trapped vibrational states in synthetic β -sheet helices†

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Received (in Cambridge, UK) 20th April 2009, Accepted 3rd June 2009

First published as an Advance Article on the web 23rd June 2009

DOI: 10.1039/b907830d

Femtosecond vibrational pump–probe spectroscopy on β -helical polyisocyanopeptides reveals vibrational self-trapping in the well-defined hydrogen-bonded side groups that is absent when non-hydrogen bonded monomers are mixed in.

In 1973 Davydov hypothesised that the energy-transport mechanism in proteins and enzymes, which remains to this date largely unknown, may actually occur by a vibrational soliton mechanism.^{1,2} It was suggested that in an α -helix, coupling between the amide I (CO-stretching) mode of the amide units and the NH \cdots OC hydrogen bonds can lead to localisation (self-trapping) of vibrational energy. The vibrational excitation would then be accompanied by a local contraction of the hydrogen-bonded chain. This self-trapping makes it possible to transport the vibrational energy along the hydrogen-bonded chain in the form of a dispersionless wave packet, which would be spread out over only a few consecutive amide units in the hydrogen-bonded chain. As a consequence, the energy generated at one site of the protein could thus be transported to another site without any dispersion of the energy packet.^{1,3}

In order to verify the soliton concept the amide I vibrational self-trapping has been extensively studied over the last decades in particular in crystalline acetanilide, a solid compound with a well-defined array of hydrogen bonds, often used as a model for α -helices.⁴ Despite this considerable interest, to date only Hamm and co-workers have reported a direct observation of self-trapped vibrational states in an α -helix.² They observed self-trapping of the NH-stretch (rather than the amide I) mode in poly- γ -benzyl-L-glutamate. Using femtosecond infrared pump–probe spectroscopy Edler *et al.* could directly monitor the self-trapped vibrational states through their anharmonicity.² Upon excitation of the NH-stretching mode, two positive excited-state bands in the pump–probe response were observed, which were attributed to self-trapped NH-stretch excitations. Interestingly, the NH-stretch mode has an energy approximately equal to that of ATP \rightarrow ADP conversion, relating it to the transport of energy in biological systems.

Here, we investigate whether NH-stretch self-trapping can also occur in β -sheet structures.⁵ To this purpose, we study the self-trapped vibrational states in synthetic β -sheet helices, *i.e.* polyisocyanopeptides. These helical polymers possess peptide side chains that form well-defined NH \cdots OC hydrogen-bonded networks, which are arranged in the form of four β -sheet type arrays along the polymer backbone. By engineering the structure of this polymer, *i.e.* by the inclusion of side arms without amide functions, the hydrogen-bonded NH \cdots OC chain in which the self-trapping occurs can be readily modified without significantly changing the secondary structure allowing the self-trapping process to be more easily quantified. Polyisocyanides can be readily prepared by nickel(II) induced polymerisation of isocyanide monomers to give helical macromolecules.⁶ The introduction of a chiral peptide substituent (*e.g.*, L or D-alanine) as side chain both stabilises the helix and induces a preferred helix handedness (Fig. 1).⁷ Dipeptide derived polyisocyanides have a typical MW of 10^6 g mol⁻¹ as analyzed by AFM⁷ and possess a highly defined rigid (persistent length of 76 nm)⁸ secondary structure in which the side arms form the above-mentioned β -sheet-like arrays of hydrogen bonds.

The resulting polymers are extremely stable and remain ordered even in competitive hydrogen-bonding solvents such

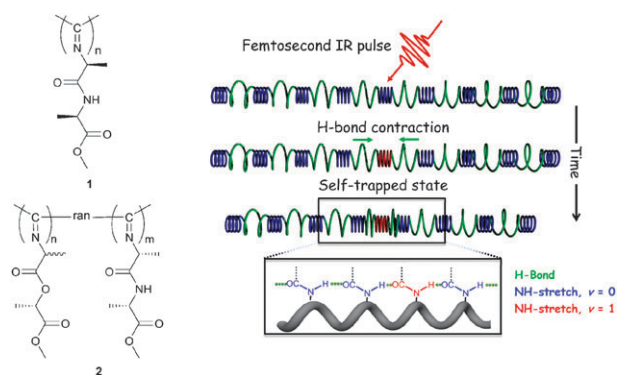
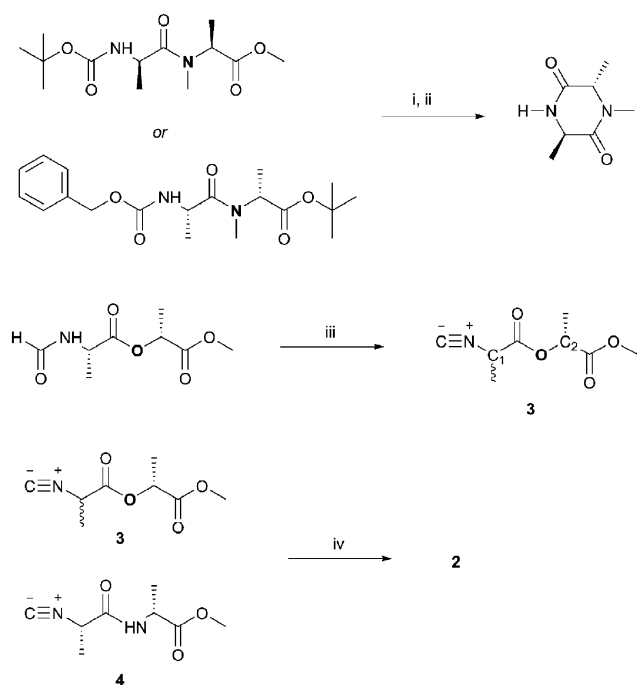


Fig. 1 (a) Molecular structure of poly-(L-isocyanolalanyl-D-alanine methyl ester) (LD-PIAA, **1**)⁷ and the random copolymer **2** synthesised by random polymerisation of D-isocyanolalanyl-L-alanine methyl ester (DL-IAA, **4**)⁷ and monomer **3** (see Scheme 1) used in this study. (b) Schematic drawing of the vibrational self-trapping process. An ultrashort (100 fs) infrared pulse is used to resonantly excite an NH bond in the hydrogen-bonded chain to the $\nu = 1$ state. This causes contraction of the hydrogen bonds attached to the amide group, which in turn causes a lowering of the stretching frequency of the excited NH bond. The excited NH-group is thus no longer in resonance with its neighbouring NH-groups. This prevents delocalisation of (hence ‘traps’) the vibrational excitation.

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† Electronic supplementary information (ESI) available: Full experimental description of the femtosecond pump–probe experiments and characterisation of the monomers and polymers used in this work. See DOI: 10.1039/b907830d



Scheme 1 Synthesis of compounds 2–4 and the formation of the diketopiperazine. *Reagents and reaction conditions:* (i) EtOAc·HCl or in the case of the *tert*-butyl ester Pd/H₂, MeOH; (ii) HCO₂Et, NaHCO₂, various conditions/2,4,5-trichlorophenyl formate, DIPEA, CH₂Cl₂; (iii) diphosgene, *N*-methylmorpholine, CH₂Cl₂, –30 °C; (iv) 0.03 equivalents Ni(ClO₄)₂·6H₂O, CH₂Cl₂ with a monomer feed ratio of 15 (3) to 1 (4).

as methanol and DMSO. Disruption of the β -sheet network of the polymer is only possible by the addition of strong acids, such as trifluoroacetic acid. This well-defined hydrogen-bonding network and stiffness makes the helical polyisocyanopeptide 1 ideal for the study of the NH-stretched self-trapped states in amino acid hydrogen bonding using femtosecond vibrational pump–probe spectroscopy.⁹ In order to further quantify the self-trapping states the random copolymer 2 consisting of monomers 3 and 4 (DL-IAA)⁷ (Scheme 1) has also been studied in which the hydrogen-bonding chain is interrupted.¹⁰ Changing the NH : O fraction in the copolymer allows one to manipulate the degree of connectivity of the hydrogen-bonded chain of NH moieties without changing the secondary structure.

The NH-stretch vibrational self-trapping was followed using femtosecond vibrational pump–probe spectroscopy. Upon excitation of the NH-stretch mode with a short (100 fs) infrared pulse (centre frequency 3260 cm^{–1}), the optical response can be observed using a time-delayed probing pulse which is detected in a frequency dispersed manner.¹¹ The result is shown in Fig. 2. The identical delay (see inset) and polarisation⁹ dependence implies that the two excited-state absorption peaks are both due to the NH-stretching mode (Fig. 2). This anomalous response (for normal vibrational excitations only a single excited-state absorption peak is observed¹²) is very similar to the one observed by Hamm and co-workers for self-trapped states in α -helices.² The two peaks represent absorptions of two-vibron bound states (TVBS) in which the vibrational energy is localised on a single

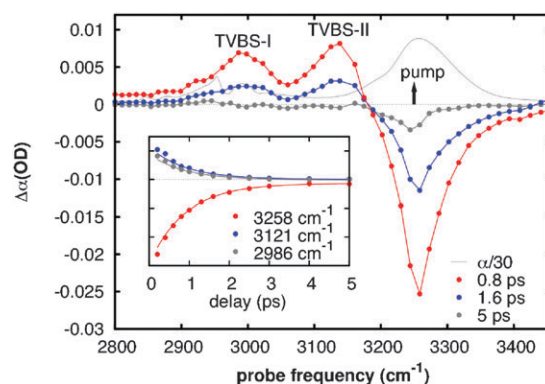


Fig. 2 Transient absorption change of LD-PIAA 1 upon NH-stretch excitation, at three delay times (see below). The thin curve represents the steady-state absorption spectrum. The inset shows the time dependence of the absorption change for three probing frequencies, together with least-squares-fitted exponential decays with time constants of 0.80 ± 0.04 ps (3258 cm^{–1}), 0.78 ± 0.11 ps (3121 cm^{–1}), and 0.73 ± 0.05 ps (2986 cm^{–1}).

NH-group (TVBS-I) and on nearest-neighbouring NH-groups (TVBS-II), respectively.²

To investigate if we could modify or change the self-trapping behaviour without influencing the secondary structure, the transient absorption change of the random copolymers was also measured. It was presumed that the hydrogen-bonding network in the latter polyisocyanopeptide side chains will be partially interrupted, which should lead to isolated NH moieties and consequently a low chance of two neighbouring NH-groups interacting with each other. We chose to incorporate a non-hydrogen bonding isocyanide (with similarities to the bis-alanine derived isocyanide) instead of denaturing the secondary structure (by acid or high temperature)², as the latter method would change the overall conformation of the polymer and lead to a complex, inhomogeneously broadened infrared spectrum. To disrupt the one-dimensional hydrogen-bonding network in the side chain initial focus was directed to the incorporation of an *N*-methylated alanine unit in the isocyanopeptide, but this however proved to be unsuccessful due to cyclisation of the *N*-methylated monomer into a diketopiperazine (Scheme 1 and ESI†). As a consequence focus was directed to replace an amide moiety with an ester functionality, as illustrated in monomer 3 (Scheme 1 and ESI†). In short, Boc-L-Ala-OH was first coupled, using carbodiimide chemistry, to methyl (*R*)-(+)-lactate methyl ester. Liberation of the free amine of the 'dipeptide' with acid followed by refluxing in ethyl formate in the presence of sodium formate generated, after column chromatography, the formamide ((*S*)-((*R*)-1-methoxy-1-oxopropan-2-yl)2-formamido-propanoate) derivative. Dehydration of the *N*-formyl moiety with diphosgene and *N*-methylmorpholine resulted in the isocyanide derivative 3 which could be subsequently copolymerised. ¹H NMR spectroscopy (Fig. S1 and S2, ESI†), however, revealed complete epimerisation of the first 'amino acid' residue, as also indicated by the doubling of the signals in the ¹³C NMR spectrum. The formamide was optically pure as evidenced by the comparison of the optical rotation of the formamide precursor of 3 ($[\alpha]_D = +17$ (c 1.2, CHCl₃)) with the enantiomer of the formamide ($[\alpha]_D = -17$ (c 1.2, CHCl₃),

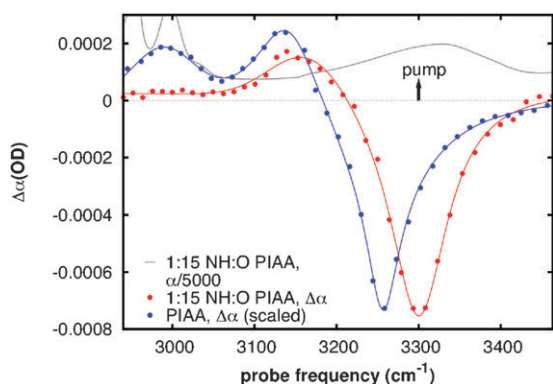


Fig. 3 Absorption change upon NH-stretch excitation of LD-PIAA and of random copolymer **2** (red line 1 : 15 NH : O). Disruption of the hydrogen-bond network completely eliminates vibrational self-trapping.

see ESI†). Epimerisation was further confirmed by chiral HPLC, which showed two retention times for the isocyanide (7.32 min and 8.29 min) (Fig. S3, ESI†). In spite of the epimerisation behaviour of monomer **3**, which would result in two helices of opposite handedness, the self-trapping could still be studied since the hydrogen-bonding network would be identical. Therefore, a random copolymerisation based on isocyanides **3** and DL-IAA (**4**) (with a 15 : 1 ratio of **3** : **4**)^{7,10} was carried out at room temperature with Ni(ClO₄)₂·6H₂O as a catalyst. The resulting polymer **2** was precipitated from diethyl ether, obtained as a powder, characterised by ¹H and ¹³C NMR and IR spectroscopy and investigated by vibrational spectroscopy (ESI†).

The pump-probe signals of the NH : O random copolymer **2** (Fig. 3), when compared to those of the regular β-sheet helix, reveal that disruption of the hydrogen-bonded NH···OC chains in the β-sheet structure leads to a dramatic change in the vibrational response. Upon disturbance of the hydrogen-bond structure the NH-stretch vibrational response becomes completely regular (exhibiting a single excited-state absorption peak) and shows no evidence of self-trapping.

Our studies show that NH-stretch vibrational self-trapping occurs not only in α-helices but also in synthetic β-sheet helical polymers. This implies that delocalisation (spreading out) of the vibrational excitation can be eliminated, which is one of the requirements for vibrational energy transport by means of Davydov solitons.^{1,2} At present, the role of Davydov solitons in energy transport in proteins is still under debate, and needs further investigations. The high persistence length of peptide-based polyisocyanides, combined with their structural integrity, allows one to further tailor their hydrogen-bonding structure, making these synthetically well-accessible polymers

excellent model systems for the study of vibrational self-trapping and energy transport in β-sheet-like structures.

Erik Schwartz would like to thank Nico Veling for providing the schematic drawing in Fig. 1. The Technology Foundation STW, NanoNed, The Council for the Chemical Sciences of the Netherlands Organisation for Scientific Research, and the Royal Academy for Arts and Sciences are acknowledged for financial support.

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